REMARKS

The Office Action of October 4, 2006 presents the examination of claims 14-34. The present paper amends claims 20 and 32, cancels claims 19 and 25-31, and adds claims 35-41. Claims 33-34 stand withdrawn from consideration.

Support for claim amendments

The amendment to claim 20 is supported by the specification, at least at page 6, line 2. The amendment to claim 32 merely rewrites the claim into independent form. New claim 35 is supported by the specification at, *e.g.* page 6, lines 3-4 (species of organisms) and by Example 2 beginning at page 15 (amplification). New claim 36 is supported by the specification generally, and Example 3 especially as to production of a luciferase from a cultured microorganism. New claims 37-40 are supported by the specification at, *e.g.* the Sequence Listing SEQ ID NOs:4, 6 and 8, which presents the sequence of a number of different luciferase proteins, taken with the knowledge of one of ordinary skill in the art who could align the various sequences to derive the common structure shown in claims 37-40. The sequences recited in the claims match residues 483 to 497 of each of SEQ ID NOs:4, 6 and 8. New claim 41 is supported by SEQ ID NOs:4, 6 and 8 and by Example 4 beginning on page 18.

Claim objections

The Examiner objects to claim 32 as depending from a non-elected claim. Claim 32 is rewritten into independent form, thus obviating this objection. Claim 18 (and 20-22) is objected to as reciting "derived from the luciferase", whereas the Examiner would prefer "derived from a native luciferase". Claims 18 and 20-22 have been so amended, thus obviating this objection.

Rejection under 35 U.S.C. § 112, first paragraph

Claims 14-24 and 32 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description support in the specification. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

The Examiner's basic position is that the claims do not impose any structural limitations on the luciferase protein molecule claimed. As a threshold matter, a composition of matter need not be limited by structure in any way. MPEP 2163(II)(3)(a) clearly states that the written description requirement can be met by disclosure of "complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics." (citing *Enzo Biochem v. Gen-Probe,* 63 USPQ2d at 1613). Thus, a description of the invention, as in this instance, by its chemical and physical properties, is an entirely adequate substitute for any structural description. Furthermore, the specification "need only describe that which is new or not conventional" (citing *Hybritech v. Monoclonal Antibodies,* 231 USPQ at 94).

The Examiner is reminded that the issue with respect to examination of adequacy of written description is whether or not the specification provides evidence that the inventors were in fact "in possession" of the invention that is claimed. *Vas-Cath v. Marhurkar,* 19 USPQ2d 1111 (Fed. Cir. 1991). Furthermore, the viewpoint of the analysis is not from a vacuum, as the Examiner seems to look from, but rather that of one of ordinary skill in the art. *Wang Labs v. Toshiba Corp.*, 26 USPQ2d 1767, 1774 (Fed. Cir. 1993).

In the present instance, the specification describes the mutant luciferases of the invention as derivatives of native luciferases known from the prior art that are mutated so as to exhibit retention of their enzymatic activity in the presence of surfactants. Examples of three such mutant enzymes that are completely structurally characterized are presented in the Sequence Listing (SEQ ID NOS: 4, 6 and 8). Furthermore, there is description in the specification of how to isolate additional mutant luciferases that retain their activity in the presence of a surfactant using site-directed mutagenesis by PCR and primers of sequences disclosed (SEQ ID NOS: 1 and 2, see Example 2). The specification further describes several organisms from which nucleic acids suitable as a template to prepare mutant luciferases of the invention using this method can be obtained.

The present claims 14-18 and 20-24 describe the invention, in terms commensurate with the description in the specification, as derivatives of known luciferases that are mutated and

retain their enzymatic activity in the presence of a surfactant. Detailed description of the structure of a native luciferase enzyme is not required, as the sequences of many native luciferases are known. *See, e.g. Capon v. Eshhar*, 76 USPQ2d 1078 (Fed. Cir. 2005). Applicants submit that the remaining description of the claimed enzyme as having at least one amino acid mutation and yet retaining enzymatic activity in the presence of a surfactant represents an adequate description of the claimed enzyme by chemical and physical properties.

Applicants submit that in view of the entirety of the specification, such description is strong evidence to one of ordinary skill in the art that the inventors in fact possessed the invention as claimed. Accordingly, the rejection of claims 14-18 and 20-24 under 35 U.S.C. § 112, first paragraph, for lack of adequate written description support, should be withdrawn.

In addition, the new claims 37-40 include further description of partial structure of the claimed enzyme, reciting a minimal amino acid sequence in the enzyme. The Examiner should also consider that this minimal sequence spans an amino acid position (490) that is deemed important to the function of the claimed protein. The inclusion of such additional structural detail to the claim, and its stated relation to function in the specification, provides an additional reason why these new claims should not be rejected.

Furthermore, case law makes it very clear that, as an alternative to describing an invention by structure, it is entirely appropriate to claim the invention in product-by-process terms. *Fiers v. Revel*, 25 USPQ2d 1601 (Fed. Cir. 1993). The present claims 32, 35 and 36 claim the instant invention in such terms and therefore, no structural limitation need be present in these claims. The Examiner may further consider that claim 35 implicitly includes some structural feature in that the sequences of amino acids encoded by the primer sequences SEQ ID NO:1 and SEQ ID NO:2 will be incorporated into the mutant enzyme as a result of the amplification process. For these reasons the instant rejection of claim 32 should be withdrawn, and the instant rejection should not be applied to new claims 35 and 36.

Claims 14-24 and 32 are also rejected under 35 U.S.C. § 112, first paragraph for alleged lack of enabling disclosure. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

In the first, instance, the Examiner makes this rejection solely upon a premise that the art is unpredictable. That is, one of ordinary skill in the art cannot predict *a priori* whether a given mutated luciferase amino acid sequence would be a luciferase enzyme that is active in the presence of a surfactant. However, the case of *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988), makes clear that this premise alone is not sufficient grounds for a rejection for lack of enablement. Rather, to establish a proper case of *prima facie* lack of enablement, the Examiner must weigh a number of factors, of which unpredictability in the art is but one. The failure of the Examiner to consider all of these factors in making the instant rejection constitutes legal error reversible on appeal.

Applicants present their considerations of the *Wands* factors below:

Nature of the invention

The invention relates to mutants of luciferases, a class of enzymes for which many examples of native purified and sequenced proteins are known in the art. The mutant enzymes retain their enzymatic activity, or much of it, in the presence of surfactants.

Breadth of the claims

The present claim 14 encompasses any mutant luciferase that retains at least 85% of its native activity in the presence of 0.1% of a surfactant. Dependent claims recite surfactants of different natures and also recite different sources of native luciferase enzymes that are mutated. The scope of these sources is narrowed systematically from families to particular organisms named by genus-species or common name. Claims 24 and 41 recite that the luciferase includes mutation of a particular amino acid. Dependent claims 37-40 describe at least a short amino acid sequence present in the luciferase.

The present claim 32 is a product-by-process claim and is similarly broad in scope to claim 14. Claim 35 is also a product-by-process claim that recites details of how mutations are introduced into the luciferase, names organism sources for a nucleic acid template used to make the mutant protein, and specifies the structure of primers to be used in an amplification method for making the mutant protein.

Level of ordinary skill in the art

The level of ordinary skill in the art is generally taken to be high. Practitioners in the art of molecular biology typically have a Ph.D. degree and often several years of post-doctoral experience. One of ordinary skill in the art has many years experience in the design and implementation of experiments related to studying the structure and function of enzymes, including techniques of screening of libraries of mutants.

Disclosure of the specification including the presence of working examples

The specification discloses that the enzymes of the invention are those mutants of native luciferases having resistance to loss of activity in the presence of a surfactant. The measurement is made in comparison to the activity of the native enzyme, which is shown to lose activity in the presence of surfactant. The amino acid residue corresponding to position 490 of the HEIKE or GENJI firefly luciferases is stated to be an important determinant of stability of activity in the presence of a surfactant. (This is residue 487 for the North American firefly luciferase, p. 7, line 8 of the specification.)

The specification also discloses two oligonucleotides SEQ ID NOS: 1 and 2, that are useful for mutating the amino acid position 490 of a luciferase. The specification further discloses two working examples of mutation of firefly luciferases at this position and also assays of luciferase activity showing retention of activity in the presence of surfactant.

A number of organisms producing native luciferases are disclosed at page 6, lines 1-4.

The specification also discloses that randomized mutation methods may be used to introduce initially uncharacterized mutations into the sequence of a luciferase (page 8, lines 1-6) and that genes encoding such mutants can be easily cloned in a library and the library can be conveniently screened by assay of luciferase activity in the presence of a surfactant (page 8, lines 6-18). Applicants note that a luciferase assay is described in Example 1 on pages 13-14 as taking from 20 seconds to 1 minute.

State of the art at the time the invention was made

At the time of filing of the present application, numerous luciferases, of both native and mutant forms, were known from a variety of organisms and had been characterized by amino acid sequence. Tools for aligning amino acid sequences of various luciferases were known, and also automated assays for luciferase were known (*e.g.* as evidence by Exhibit 1). As the Examiner admits, techniques for introducing mutations throughout an amino acid sequence were also known. Applicants submit that, contrary to the Examiner's position, methods for introducing multiple mutations in a single sequence were indeed known, indeed commonplace, at the time of filing. Examples from the art showing random mutagenesis to introduce multiple mutations in a protein sequence and subsequent screening for activity are provided in Exhibits 2 and 3.

Amount of experimentation to practice the invention

The amount of experimentation needed to practice the invention is not large. A few days may be required to make a library of luciferase mutant genes and get them expressed in bacteria or yeast cells. As indicated above, robotic assay of luciferases was known, and so large-scale screening of such mutant libraries was feasible at the time of filing of the application. Comparison of activity of a large number of mutant enzymes to a native enzyme in the presence or absence of surfactants of various natures and concentration may take a few weeks at most.

Predictability in the art

The Examiner asserts that the art is unpredictable. It is true that one of ordinary skill in the art may have some difficulty predicting *a priori* which mutations in a luciferase protein will result in retention of activity in the presence of surfactant. However, that is not the kind of predictability that is at issue. Rather, the issue is, given a one or more embodiments of the present invention, is it predictable that the skilled artisan can utilize the teachings of the specification together with his knowledge of the art to produce further embodiments spanning the scope of the claims. In the instant case, because of the ease of making large libraries of mutations and testing them for activity under various conditions, the art must be said to be predictable, as it is more likely than not that the practitioner can in fact find a large number of operable mutants.

The Examiner is further reminded that it is not necessary that every experiment produce the desired result, *i.e.* not every mutation made and tested need work. In the *Wands* case, only 2.5% of the hybridomas screened secreted the desired antibody, and several of the screening experiments conducted failed to reveal any desired hybridomas. Nonetheless, the invention was deemed to be enabled by the Court of Appeals for the Federal Circuit.

The Examiner is further reminded that the issue is not whether experimentation is necessary to practice the invention broadly. Rather the question is whether such experimentation that is needed is undue. Experimentation that is expected to be conducted, and that is guided by the specification or state of the art, is not undue experimentation. See, *Wands*.

Applicants submit that a proper weighing of the factors to be considered in assessing enablement will result in withdrawal of the instant rejection.

Rejection over prior art

Claims 14-21, 23, 24 and 32 are rejected under 35 U.S.C. § 102(e) as being unpatentable over Hirokawa et al. '859. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

Hirokawa discloses improvement in activity of luciferases under varying conditions of pH. Hirokawa makes no disclosure whatsoever regarding stability of the enzyme in the presence of surfactant. Activity assays appear to be performed in amine sulfonate buffers such as CHES, TAPS and MES (cols. 12-13). Hirokawa does not ascribe any surfactant activity to these buffers and no additional surfactant is disclosed as added to the assays. The Examiner does not show that these buffers have any surfactant activity, especially at the 100 mM concentration (approximately 2% w/w given a molecular weight of 200 g/mol) stated. In any event, the amount of these buffers in the assay is well above the 0.1% amount of surfactant recited in the claims. Thus, Applicants submit that the buffers disclosed by Hirokawa are not "surfactants" within the meaning of that term in the present application.

Applicants are not certain whether the Examiner is relying upon a theory of inherency, but if so, note that the Examiner admits that there are a number of differences in amino acid sequence between the luciferases disclosed by Hirokawa and the luciferases in the examples of the present application. As such, the Examiner is reminded that inherency requires that exact identity of the luciferases would be required to assert with confidence that the luciferases of Hirokawa would necessarily, not merely possibly, or even likely, retain their activity in the presence of surfactants. This is especially so in view of the Examiner's position that retention of biochemical characteristics of an enzyme in the presence of mutations is unpredictable.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Mark J. Nuell Reg. No. 36,623 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

Dated: April 4, 2007

Respectfully submitted,

By _____

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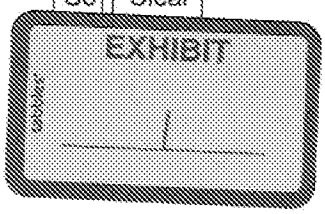
Attachments: Exhibits 1-3



1: J Appl Biochem. 1983 Jun;5(3):197-209. Links

Firefly and bacterial luminescence: basic science and applications.

McElroy WD, DeLuca MA.

The basic chemistry of the reactions leading to light emission in the firefly and in bacteria are briefly reviewed. With excess firefly reagents, the light intensity is proportional to the ATP concentration. For this reason, the reagents have been used for ATP determination in a number of important biological systems. A number of such applications are reviewed. With excess bacterial reagents, the light intensity is directly proportional to the reduced pyridine nucleotide concentration (NADH or NADPH). The applications of this system for studying reactions involving dehydrogenases using NAD or NADP as electron acceptors are presented. Many assays have now been developed using enzymes immobilized on Sepharose. The advantages of using the immobilized enzymes are greater stability of the immobilized enzymes over the soluble forms; increased sensitivity of detection relative to the soluble forms, and reusability of the immobilized enzymes. A comparison of the immobilized bioluminescent assay for 7 alpha-hydroxysteroid with gas-liquid chromatography and radioimmunoassay is presented. Coimmobilized enzymes can be packed in a flow cell and used in an automated instrument with good reproducibility. It is likely that future developments of bioluminescent assays for ATP or NAD(P)H will be with immobilized enzymes using an automated instrument.

PMID: 6680120 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A. 1996 Apr 16;93(8):3630-5.

Multidrug resistance proteins QacA and QacB from Staphylococcus aureus: membrane topology and identification of residues involved in substrate specificity.

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The closely related multidrug efflux pumps QacA and QacB, from the bacterial pathogen Staphylococcus aureus, both confer resistance to various toxic organic cations but differ in that QacB mediates lower levels of resistance to divalent cations. Cloning and nucleotide sequencing of the qacB gene revealed that qacB differs from qacA by only seven nucleotide substitutions. Random hydroxylamine mutagenesis of qacB was undertaken, selecting for variants that conferred increased resistance to divalent cations. Both QacA and the QacB mutants capable of conferring resistance to divalent cations contain an acidic residue at either amino acid 322 or 323, whereas QacB contains uncharged residues in these positions. Site-directed mutagenesis of qacA confirmed the importance of an acidic residue within this region of QacA in conferring resistance to divalent cations. Membrane topological analysis using alkaline phosphatase and beta-galactosidase fusions indicated that the QacA protein contains 14 transmembrane segments. Thus, QacA represents the first membrane transport protein shown to contain 14 transmembrane segments, and confirms that the major facilitator superfamily contains a family of proteins with 14 transmembrane segments.

PMID: 8622987 [PubMed - indexed for MEDLINE]

1: Biochem Biophys Res Commun. 1996 Jan 5;218(1):254-9. ELSEVIER Links

Analysis of p16INK4a and its interaction with CDK4.

Yang R, Serrano M, Slater J, Leung E, Koeffler HP.

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The interaction between cyclin-dependent kinase 4 (CDK4) and its inhibitor p16INK4a (p16) was studied by random mutagenesis and yeast two-hybrid system. The gene encoding p16 was mutagenized randomly and the amino acid changes that affect the binding of p16 to CDK4 were identified. Several amino acid residues were shown to be important for the binding and many of these changes occur at residues conserved in all known human p16 family proteins Most of the mutant p16 proteins that failed to bind to CDK4 contained multiple amino acid changes, and these alterations were observed throughout the entire gene with no apparent mutational patterns or hot spots. Some of the mutations that moderately reduced the binding activity severely affected the kinase-inhibitory activity of p16.

PMID: 8573142 [PubMed - indexed for MEDLINE]